CHANGES IN WEIGHT, VOLUME AND OXYGEN CONSUMPTION DURING REORGANIZATION AND REGENERATION IN *Sabella pavonina* Sav.

By

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Introduction.

Various authors (Vaney and Conte,1906; Iwanoff,1908; Berrill,1931; Berrill and Mees,1936a, b.; Huxley and Gross,1934; Gross and Huxley,1935;) have established the fact that in Serpulid and Sabellid worms head regeneration is usually combined with structural changes of original segments. The parapodia of some of the original segments degenerate and are after a time reformed into parapodia of a different type.

This reorganization was extensively studied in Sabella pavonina Sav. (Berrill,Huxley and Gross, etc.). The normal stucture of this polychaet worm and the whole process of reorganization has been described in the above mentioned papers and only a short description is given here.

The worm has essentially three different regions(Fig. 1). First the head region,which consists of two segments, the head- and the"collar"- segment. The head possesses two groups of ~~tentacles~~, each having a number of coloured spots.

morphologically palps

The second region, the thorax, consists of a number of"thoracic segments", of which each has a pair of parapodia possessing an uncinigerous lobe with uncini and a setigerous lobe with setae. The uncinigerous lobes are situated ventrally and the setigerous lobes dorsally.

The next region - the abdominal region - consists of a large number (up to 3oo) of segments of the "abdominal type", in which the uncinigerous lobes are situated dorsally and the setigerous lobes ventrally. Only the last segments is shaped differently and forms a pygidium.

On the ventral side of the abdominal region in the median line is a ciliated groove, which turns dorsal in the intersegment between the first abdominal and the last thoracic segment and continues dorsally in the thoracic region. This gives the impression as if the whole of the thoracic region had been twisted around at 180°.

Abdominal segments are able to regenerate both at the anterior and posterior cut surface. But while posteriorly a large number of abdominal segments can be regenerated, the anterior regeneration is limited. Only three segments: the head-, the "collar" , and one thoracic segment are regenerated.

The process of this anterior regeneration is as follows: a day or two after the fragment is cut the wound closes and several

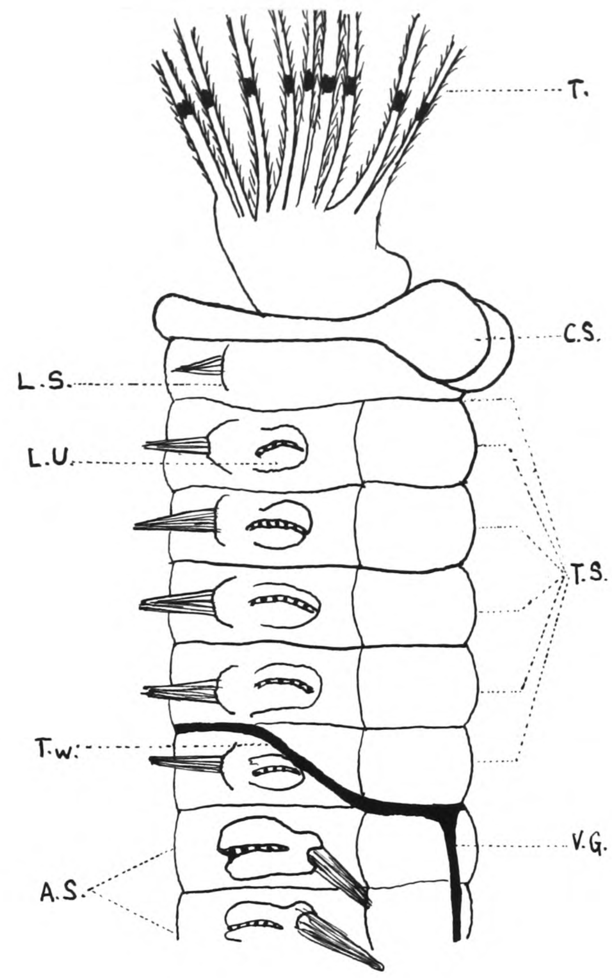


Figure 1.

A.S. abdominal segments; C.S. "collar" segment; L.S. setigerous lobe; L.U. uncinigerous lobe; T. head segment with tentacles; T.S. thoracic segments; V.G. Ventral groove; Tw. Twist of the ventral groove.

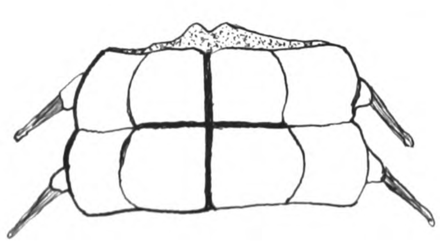


Figure 2.
Stage I.

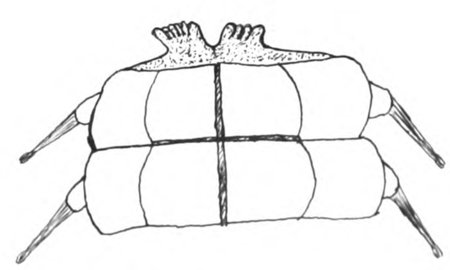


Figure 3.
Stage II.

~~several~~ days later the first rudiments of head regeneration appear in the form of two lobes (Fig.2). The two lobes develop into the head and the tentacles (Fig. 3). Then the first thoracic segment is formed and the tentacles increase in size (Fig. 4). The next stage is the full differentiation of the "collar" segment,the head segment and the appearance of coloured spots on the tentacles (Fig. 5). With the appearance of uncini and setae on the thoracic-, and of setae only on the "collar" segment the process of head regeneration is completed.(Fig. 6).

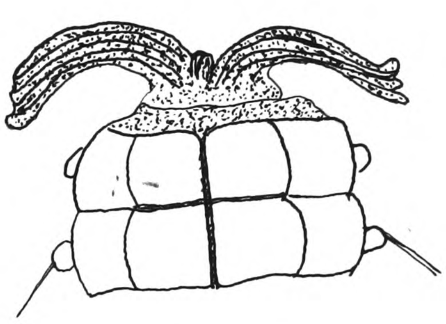


Figure 4.
Stage III

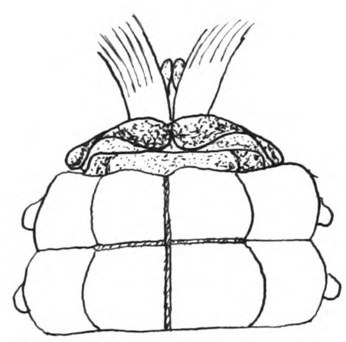


Figure 5.
Stage IV.

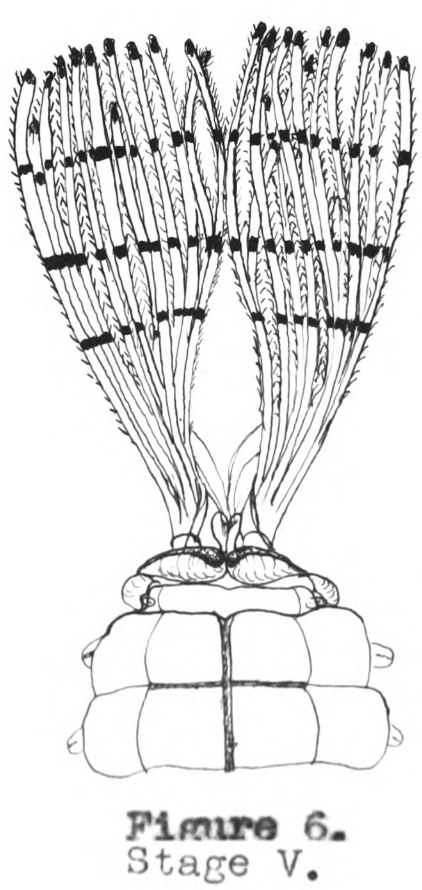


Figure 6.
Stage V.

While the regeneration appears to be limited to three segments, a varying number of abdominal segments can be transformed into segments of the thoracic type. This process of reorganization begins with the loss of uncini in the original segments, starting in the first segment and gradually proceeding posteriorly; (Fig. 7). The second stage of reorganization is characterised by the gradual degeneration of the unciniгerous lobes and the loss of setae in the segments affected, also beginning with the most anterior segment and spreading posteriorly. Simultaneously (at room temperature, 18°-20° C.) the first sign of newly formed setae appears dorsally, in the form of minute bristles (Fig. 8). All those segments, which had dropped uncini also lo~~o~~se their setae and form new ones on the dorsal side. Subsequently new uncini are formed first in the most anterior segments (Fig. 9) and later in all segments affected.

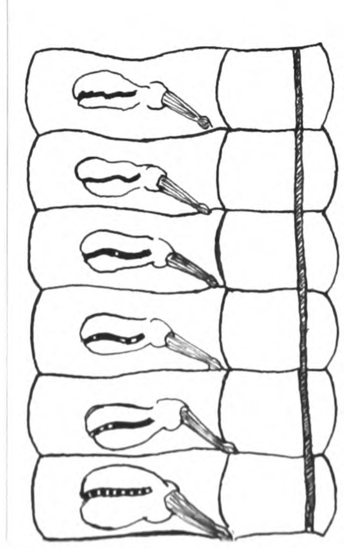


Figure 7
Stage A

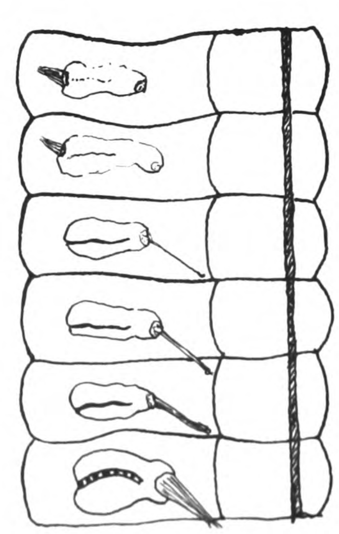


Figure 8
Stage B

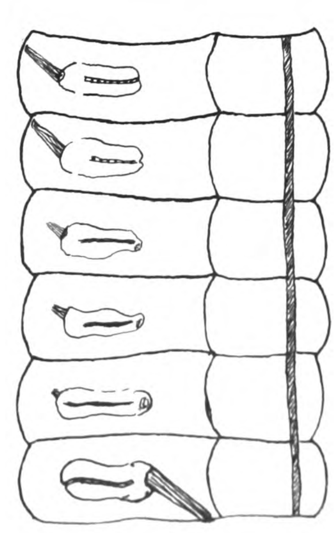


Figure 9
Stage C

In the following account the successive stages will be indicated by I---V (Figs.2---6) for head regeneration, and stages of reorganization by A--C (Figs.7--9) and D (indicating the completion of reorganization), except in fragment 102 (p. 26) where stages B_1 and B_2 are distinguished.

Interpretations given by Berrill (1931), Gross and Huxley (1935), and Berill and Mees (1936a;b.) of the morphogenetic processes involved in the process of regeneration and reorganization in Sabella pavonina will be discussed on page 33. In general very little is known of the physiological processes underlying regeneration in invertebrates. It was thought that Sabella might be suitable material for investigations on physiological lines. In this paper an account will be given of changes in weight, volume, and oxygen consumption of regenerating fragments, and an attempt made to correlate those changes with the successive stages of regeneration and reorganization.

I am indebted to Professor James Ritchie for providing me with excellent laboratory facilities in the Department of Zoology, and for his constant interest. Also to Dr. F. Gross for his encouragement, interest and his willingness to assist me in this work.

Material and Methods.

The animal material was collected at the Marine Biological Laboratories at Plymouth and Millport and sent to Edinburgh in glass-jars. The animals were kept in aerated tanks and remained in good condition for several months.

After cutting the fragments with sharp scissors they were washed twice and placed singly in shallow glass vessels (crystallising dishes) filled with "Erdschreiber solution" (Gross,1937;). The medium was changed every second or third day.

A torsion balance (1ooo mg,divided in 500 parts) was used for weighing the fragments. The excess liquid was rapidly removed with filter paper and the fragments placed in the noose of a silk thread and then weighed.

By using a torsion balance the fragments could be weighed in approximately 3o seconds, and they could therefore be weighed thoroughly dry, as they remained in this dry condition for only such a short period. The accuracy of the measurements appeared to be quite satisfactory, as the differences, found in 10 consecutive weighings of the same fragment did not exceed ± 1 mg.

The volume of the fragments was determined by weighing them submerged in sea-water.

From the loss of weight the volume could be calculated after the formula:

$$v = \frac{g_1 - g_2}{s} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

in which g_1 is the actual weight of the fragment, g_2 the weight in seawater and s the specific gravity of sea-water.

The specific gravity of sea-water was found by weighing samples in a specific gravity bottle. An average of 31 independent weights was finally taken as specific gravity (1·025) and no temperature correction applied.

The volume of the fragments determined ~~with~~ by this method sufficed for the purpose of using it in the determination of the oxygen consumption, but was not accurate enough for establishing any changes in the specific gravity of the fragments. The reasons were: the presence of small air-bubbles between setae and a certain amount of sea-water absorbed by the silk thread, which increased the error to ± 1·5 mg, i.e. more than 10% of the weight of the fragments in sea-water.

For the determination of the oxygen consumption a Micro-Winkler apparatus (Fox and Wingfield, 1938;) was used. The following procedure was adopted: the fragments were placed in weighing bottles - each fragment in the same bottle during the whole experiment -; the bottle was then filled with sea-water which had been kept at a constant temperature of 20°C to avoid gas bubbles, and then placed for an hour in an incubator kept at 20°C. During this period the bottle was shaken several times, then a sample of the water taken and the oxygen contents determined.

Simultaneously a sample of sea-water without a fragment was kept under the same conditions and its oxygen contents measured.

The oxygen consumption was calculated per gramm on the basis of a modified formula of Fox and Wingfield (1938):

$$O_2 = \frac{\dfrac{X \cdot n \cdot i \cdot 11.2 \cdot 1000}{v \cdot t}}{w} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

were x is the volume of the weighing bottle minus the volume of the fragment;

n the amount of sodiumthiosulphate used for the titration of the control-sample minus the amount used for the titration of the sample in which the fragment had been placed;

v the volume of the sample whose oxygen content was determined;

$\frac{i \cdot 11.2}{t}$ the normality of the sodiumthiosulphate solution used for the titration (see Fox and Wingfield,1938;)

w the weight of the fragment.

The normality of the sodiumthiosulfat solution (a ca. $n = \frac{1}{40}$ solution has been used) was controlled twice a week by titration against potassium iodate solution of known mol. concentration. The results obtained by this method varied by $\pm$ 3%.

The morphological changes have been ~~controlled~~ observed under a binocular microscope every 3rd day and measurements of weight,volume and oxygen consumption made daily or every 2nd or 3rd day. The animals were not kept at constant temperature except before a determination of the oxygen consumption. Experiments at low temperature were carried out during winter when some of the fragments were placed in a cold room. Temperatures referred to are therefore average temperatures.

Weight, Volume and Oxygen consumption during reorganization.

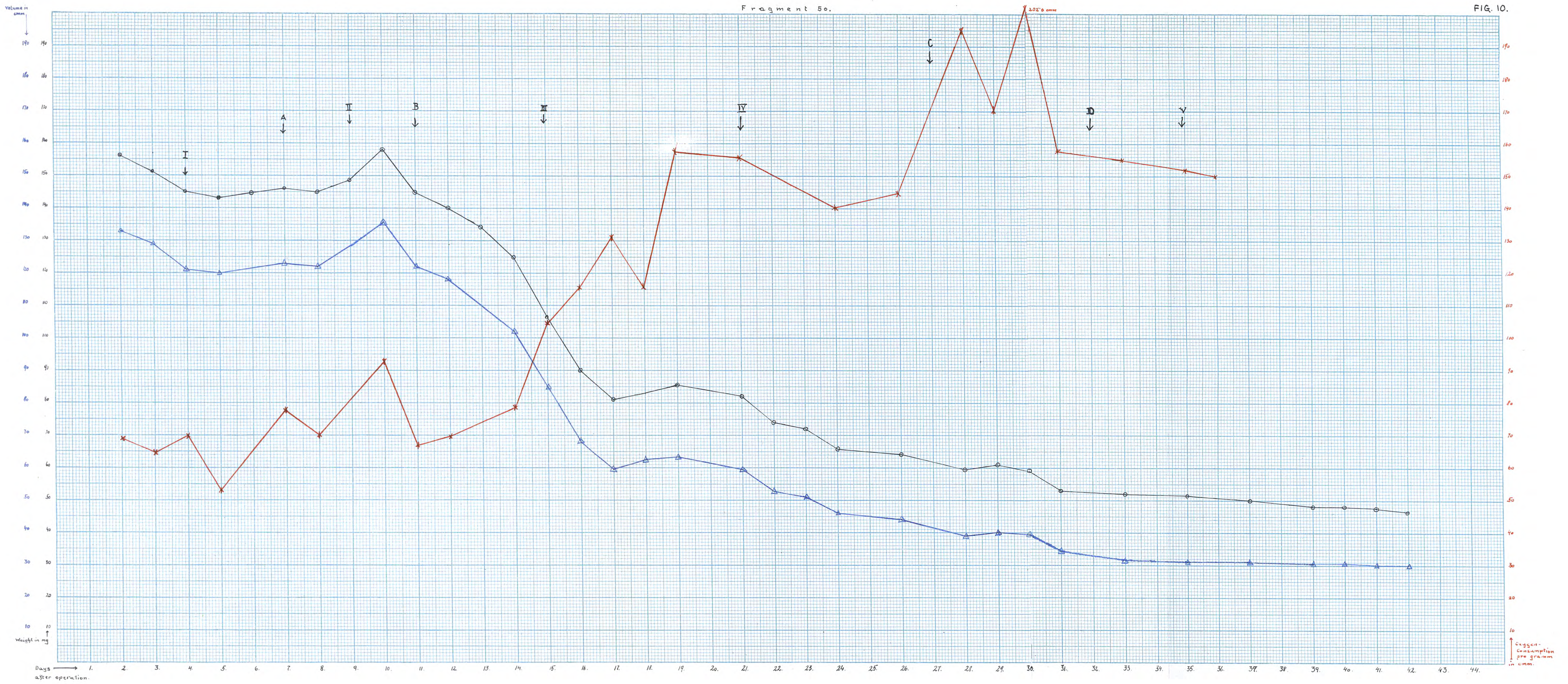
a) at room temperature (18^{0}-20^{0}C.)

After cutting, the fragments usually lose weight; this decrease in weight is probably caused by loss of blood and body fluid. After the wound closes no further loss in weight takes place. On the contrary, the weight increases again till it reaches a maximum. This period of increasing weight coincides approximately with the appearance of the first sign of reorganization, i.e. the loss of uncini.

Shortly before or after the maximum is reached setae beginn to drop out and then a very sharp decrease in weight follows. In some fragments 60% of the total weight was lost within the comparatively short period (7-10 days) of rapid decrease. After that the rate of decrease in weight of the fragments is slowed down and even slight increases could be observed now and then. After completion of the reorganization the weight still decreases slowly, probably as an effect of starvation.

During the first period of increase in weight - after wound healing - the rate of oxygen consumption ~~goes up and down~~ fluctuates. But the subsequent period of rapid loss in weight is cha-

FIG. 10.


Fragment 50.
Volume in cmm
Weight in mg
Days after operation
Oxygen-consumption pro gramm in cmm.
I
II
III
IV
V
A
B
C
D
202·6 cmm

racterised by a very sharp and marked rise in oxygen consumption. It reaches values almost three times as high as at the beginning of the experiment and remains for the whole time of growth of new parapodia, uncini, and setae on a level far above the original. It decreases slowly after completion of reorganization.

Details of the changes in weight, volume and respiration, and references to morphological changes which took place at the same time, are ~~may be~~ given in the following records and accompanying figures.

<u>Fragment</u> <u>50</u> (Fig. 10); 22 segments; operated 16.6.1939; weight at the 2nd day after cutting 156 mg, decreases to 143 mg on the 5th day, first stgge of regeneration (stage I). Oxygen consumption per gramm/hour decreases from 69 cu.mm (2nd day) to 53 cu.mm (5th day).

7th day: weight increased slightly / 146 mg/, oxygen consumption 77.5 cu.mm; uncini of the first and second segment lost (stage A).

9th day: weight 148,5 mg: first signs of tentacles.

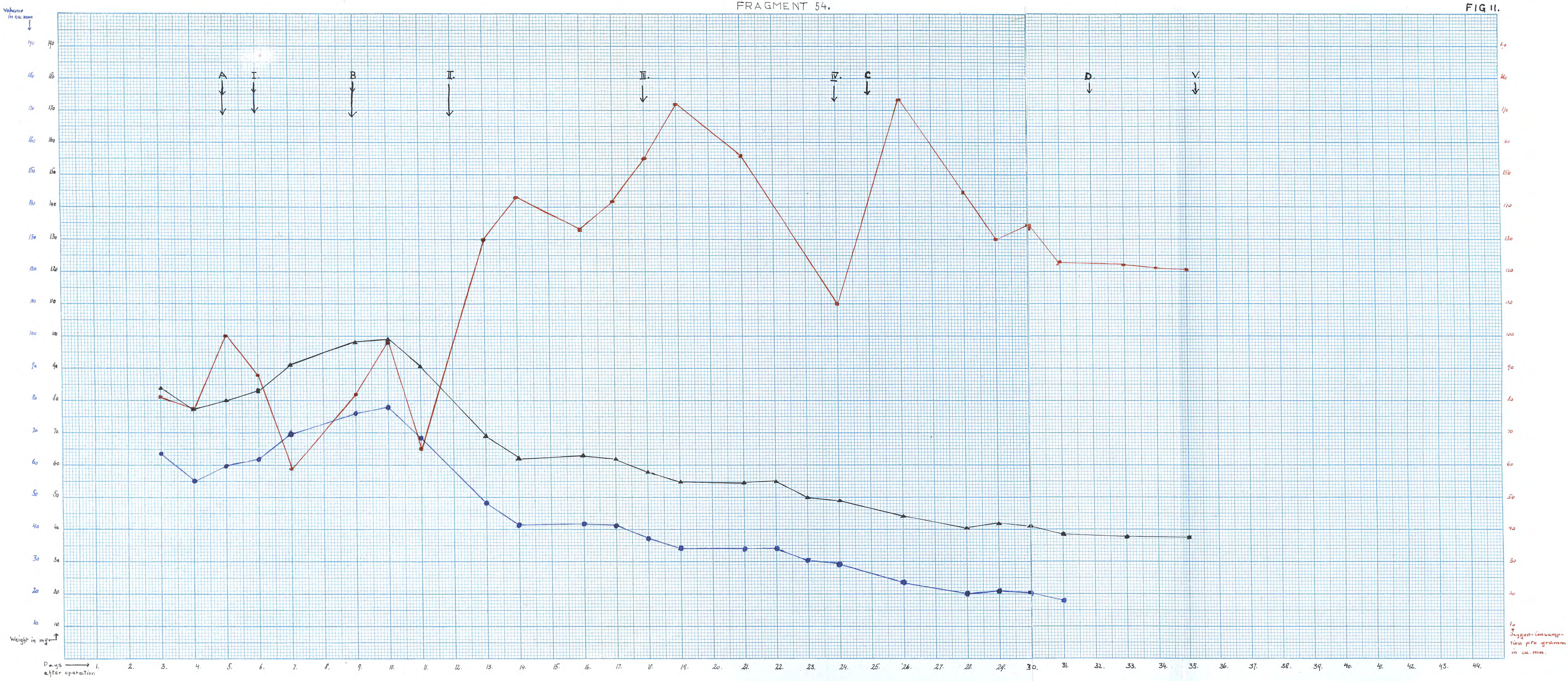
10th day : weight 158 mg, oxygen consumption 93 cu.mm; uncini lost in 5 segments.

11th to 17th day: sharp drop in weight /158mg to 81 mg; i.e. ca. 50%/; uncini of the 6th segment lost. On the 11th day setae began

to drop out and by the 17th day 6 segments had completely lost their setae.
During the same period the respiration increased considerably to 1o5 cu.mm on the 15th, 131 cu.mm on the 17th and 153 cu.mm on the 19th day. New setae were formed (stage B.) The stages IIIand IVI of regeneration were also reached during that period.
After the 19th day the rapid loss of weight and the steep increase of oxygen consumption ceased. Between the 2oth and 26th day the weight decreased slowly. The oxygen consumption also fell slightly. Weight on the 26th day64 mg, oxygen consumption 145 cu.mm.
In this period the setae grew longer and new setigerous lobes became more distinct.
During the next period (26th-30th day), while the weight was still decreasing, the oxygen consumption rose very steeply to a maximum of 202,6 cu.mm; this rise may be correlated with the processes leading to the formation of new uncini.
Then the rate of oxygen consumption fell again to 158 cu.mm (31st day) and, like the weight, continued to decrease slowly. On the 32nd day the reorganization reached completion (stage D) and on the 35th day the 3 regenerated segments were also completed

FRAGMENT 54.

FIG. II.



(stage V).

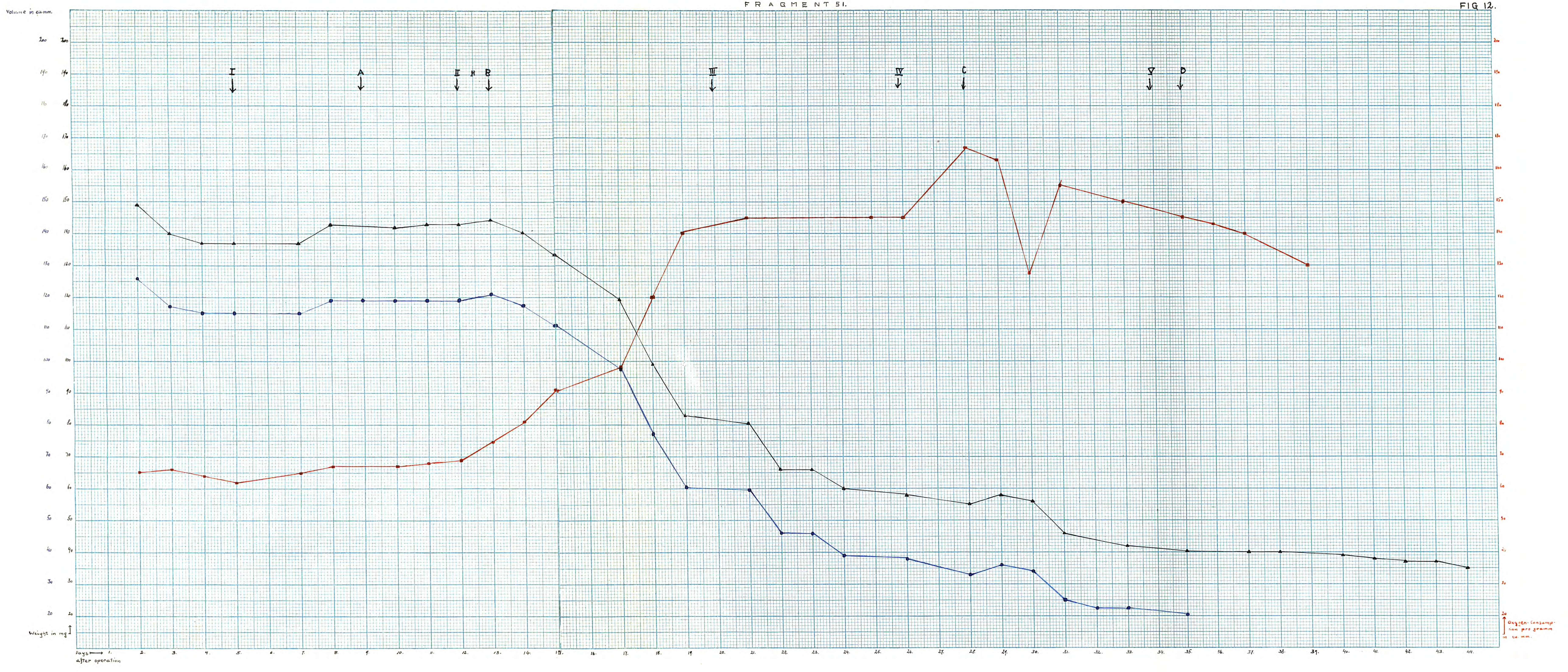
The ~~o~~curve of volume during the whole period followed closely that of the weight. It increased with increasing weight and decreased with it.

Similar was the development in

Fragment 54 (Fig. 11); 11 segments; operated 16.6.1938.; weight on the 3rd day 84 mg. Here also, after a decrease in weight to 77.5 mg (4th day) an increase takes place, reaching 99 mg on the 10th day. During this period the uncini are lost and the first signs of regeneration appear (stage I). With the beginning of the loss of setae (stage B) a very sharp decrease of weight takes place (to 63 mg, i.e. ca 45% on the 14th day) and a very marked increase in the oxygen consumption, coinciding with the appearance of new setae. In this period stage II is reached (12th day). Then a period of slowly decreasing weight follows, but the oxygen consumption reaches 172.1 cu.mm on the 19th day. Stage III falls in this period (18th day). Then, after a sharp decrease, the oxygen consumption rises again to a maximum (26th day, 173.4 cu.mm) coinciding approximately with the appearance of new uncini (stage C, 25th day).

FRAGMENT 51.

FIG. 12.



On the 31st day new uncini have been formed in 5 segments and stage D has been reached. Weight and oxygen consumption are slowly decreasing. Stage V was reached on the 35th day.

Here again the changes in volume conform closely to those of weight.

It may be noted that the completion of regeneration was delayed by about three days as compared with fragment 50 (Fig. 10), while reorganization in earlier stages (A and B) took place more speedily.

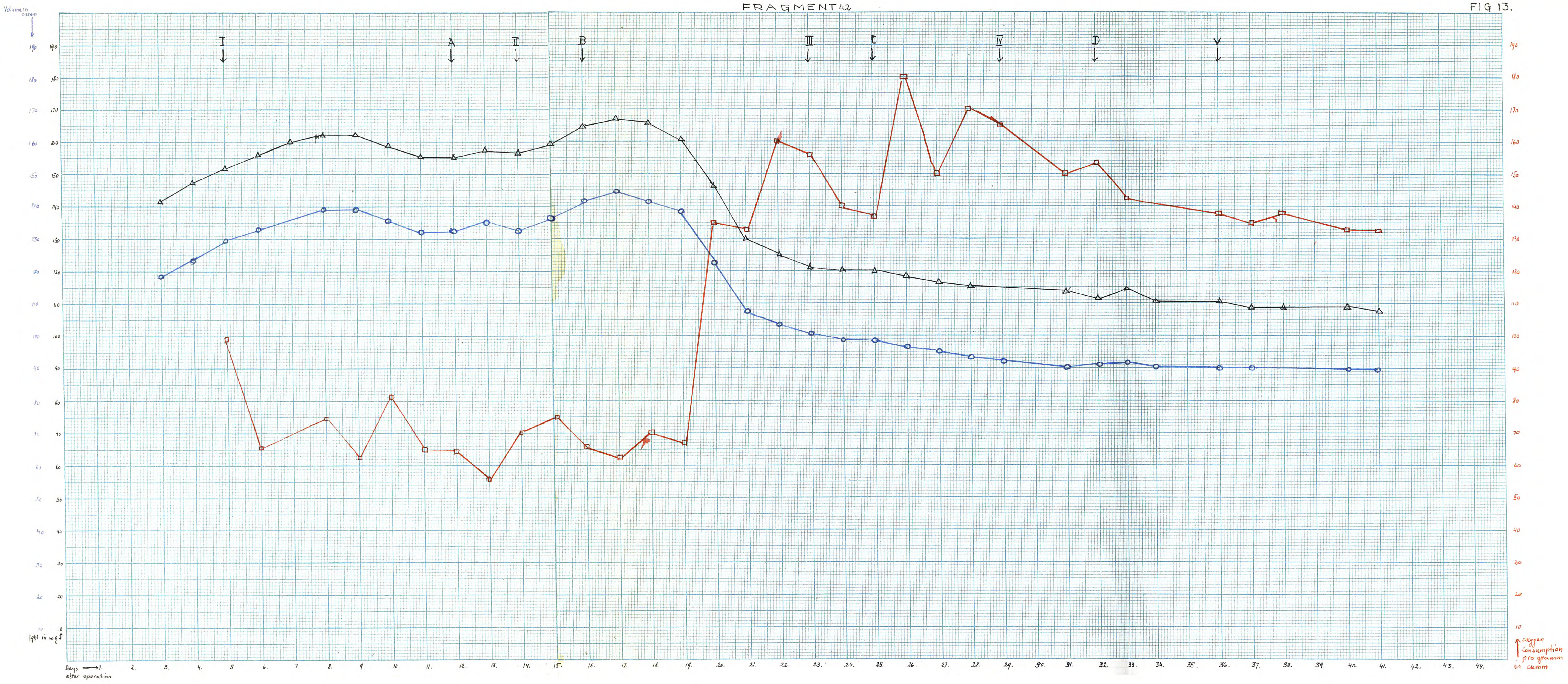
The following records show a somewhat different development of fragments; they are representative of 39 fragments investigated.

Fragment 511 (Fig. 12); operated 12.6.1939; 149 mg on the 2nd day, decreased to 137 mg on the 7th day, increased again to 144,5 mg on the 13th day, when the loss of uncini was complete in 5 segments and of setae in the 1st segment.

Then followed a period of rapid decrease in weight, (to 80.5 mg = loss of 45%) between the 13th and the 21st day and an increase in the respiration (from 74,5 cu.mm to 145 cu.mm); the setae of 5 segments dropped out and new setae appeared. During the same period stage III of regeneration was reached.

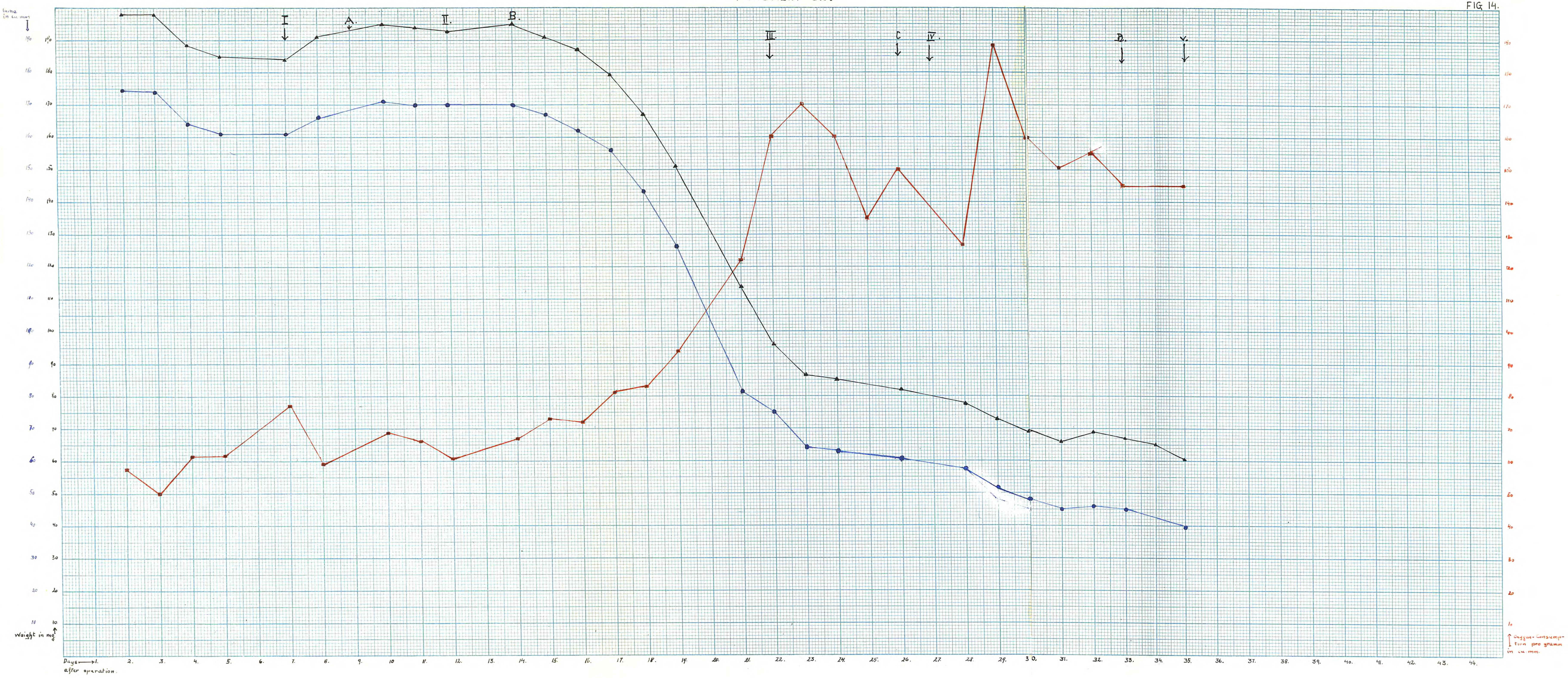
FIG 13.

FRAGMENT 42



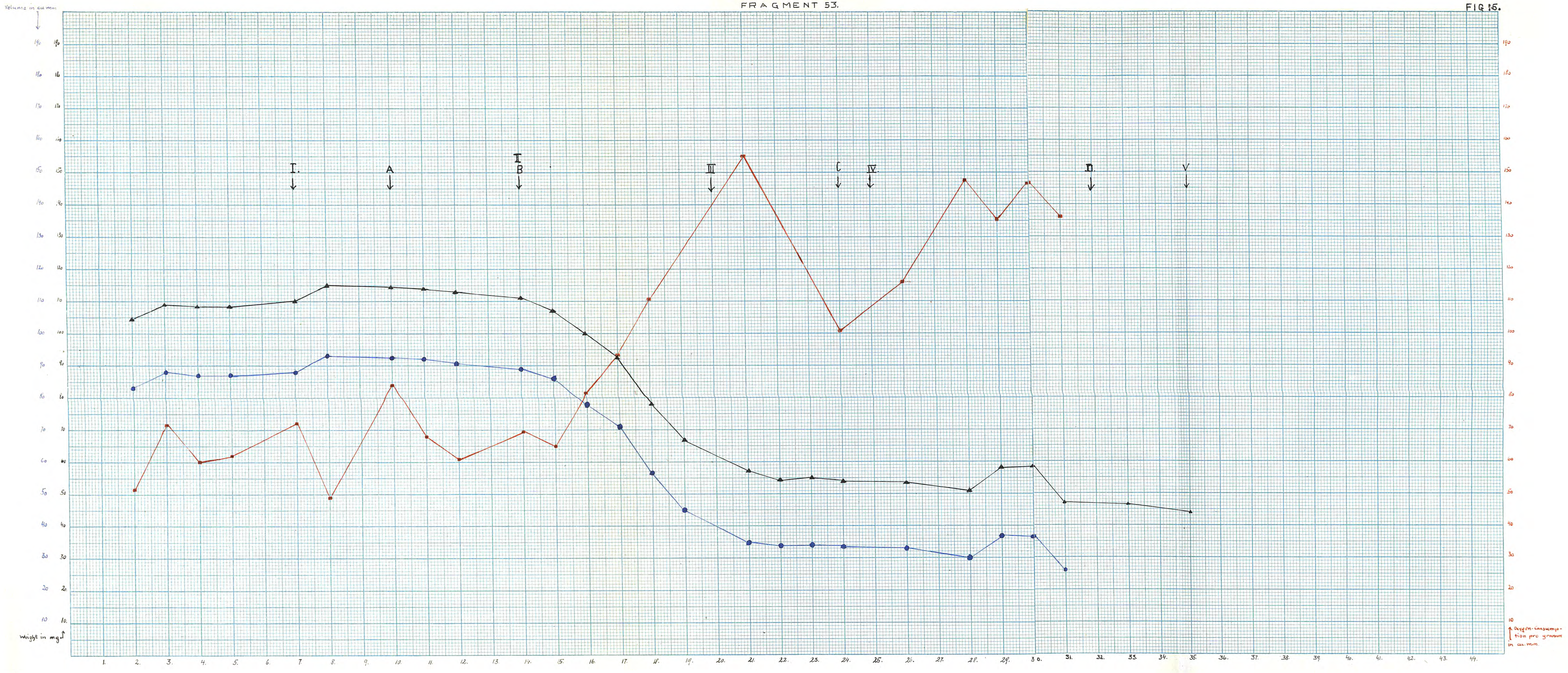
FRAGMENT 52.

FIG. 14.



FRAGMENT 53.

FIG. 15.



After a time of continual slow decrease in weight and a continually high rate of oxygen consumption the respiration rose again to 167 cu.mm on the 28th day and new uncini were formed in 3 segments.

By reaching stage V of regeneration and stage D of reorganization on the 34th and 35th day respectively the whole process was concluded.

Similar results were obtained in 38 other experiments, the result of three of them are given in figures 13, 14, 15, representing fragments 42 (21 segments, 4 segments reorganized);52 (28 segments,7 reorganized);and 53 (11 segments, 5 reorganized).

The difference between fragments of the type of fragments 50 or 54 (Figs. 10 and 11) and those of the type of fragments 51,42,52, or 53 (Figs.12, 13,14,15.) lies in a more rapid development in earlier stages and a slower development in the later stages of reorganization in fragments of the first type.

It may be pointed out that all fragments of the first type (9 such cases investigated) were parts of the region lying immediately behind the thorax of the original animal,while fragments from posterior regions follow a development described in fragment 51 (Fig. 12). This may be seen from Fig. 16, which shows the changes in weight, volume and respiration in three fragments of the same animal, of which one (fragment 50) was cut from the region lying behind the thorax and the two others (fragments 51 and 53) from a more posterior region.

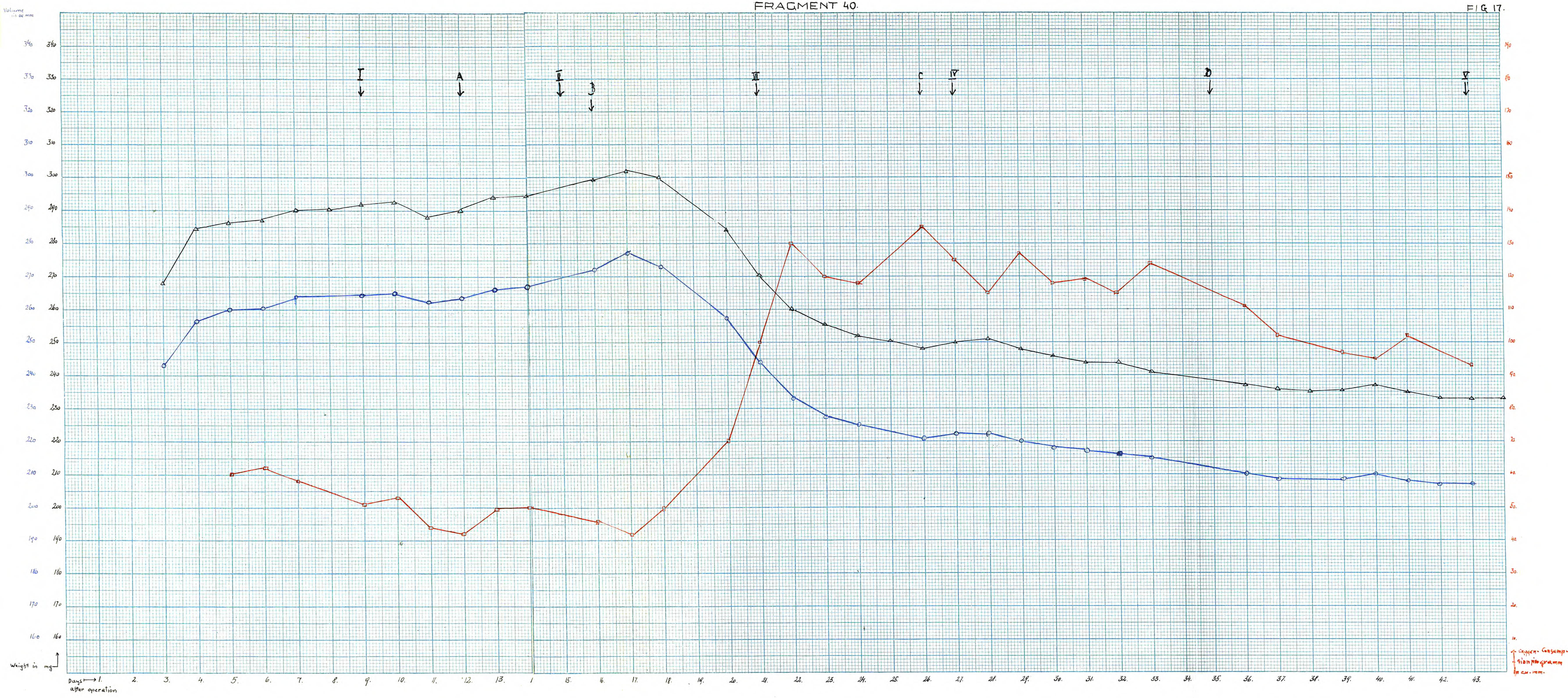
It may be added that, while no ap~~p~~arent difference in the speed of regeneration could be observed between anterior and posterior fragments, the tentacles of the first tend~~s~~ to be shorter and less developed.

The changes in weight and respiration, described in the foregoing pages were characterised by two facts : a very rapid loss (up to 60%) of the total weight during a short period (7 to 10 days) after a more or less marked increase and a steep rise of respiration (60 cu.mm to 200 cu.mm) during the same and the subsequent period, coinciding with the loss of original setae and the formation of new setae and uncini.

The regenerating fragments, described below, differ from those mentioned above in several respects. The loss of weight following the initial, more or less marked increase in weight was far smaller, so that in some instances the weight after the whole process of regeneration and re-

FRAGMENT 40.

FIG 17.



reorganization was found to be nearly the same as it was at the beginning of the experiment.

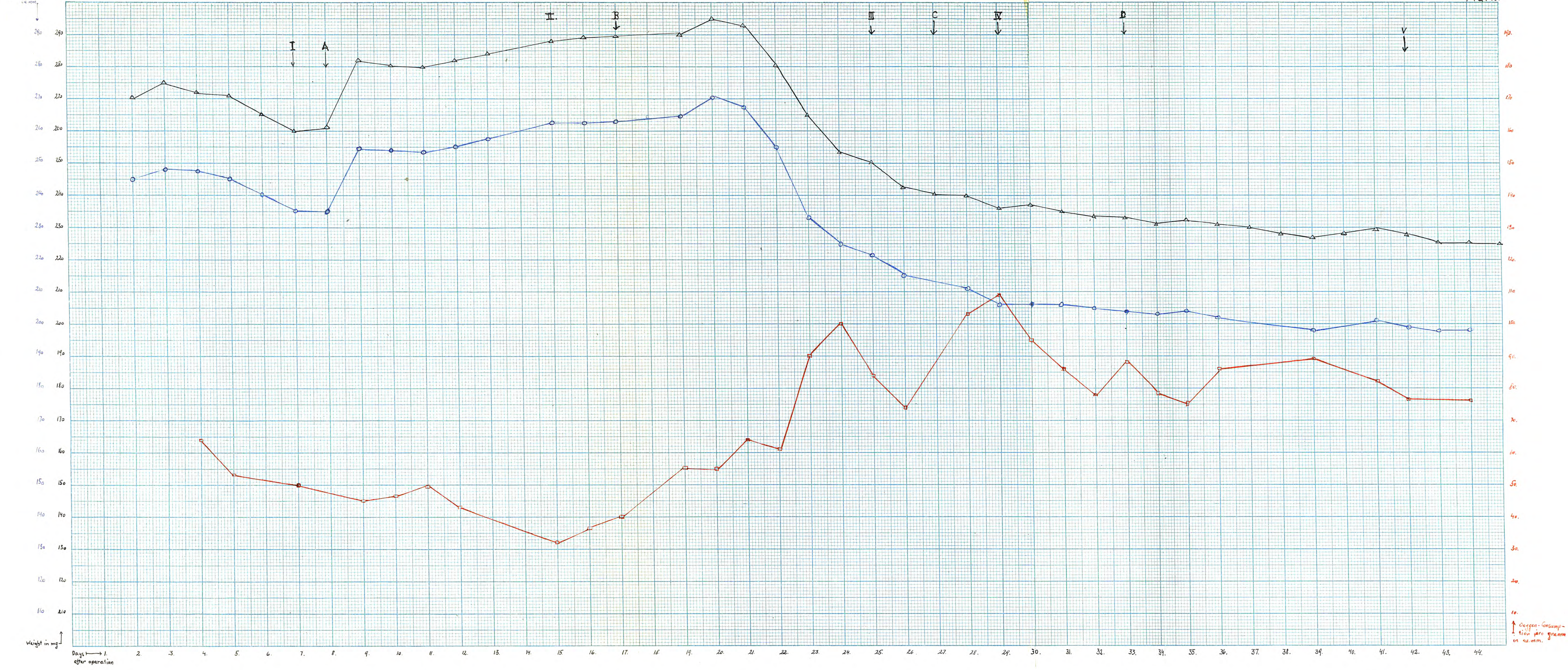
The rate of respiration did rise during the period between stage II and III and B to C respectively, but never as high as in the foregoing experiments.

This was observed with certain variations in 28 fragments, 4 of which may be described more in detail.

Fragment 40; (Fig. 17); 32 segments, operated 12.4.1939. Weight after 2 days 268 mg. Signs of regeneration appear on the 9th day (stage I); loss of uncini starts on the 12th day and continues to the 19th day, when uncini in altogether 4 segments were lost. There was no initial loss in weight; on the contrary, the weight increased from 268 mg on the 2nd day to 302 mg on the 17th day.
On the 16th day the setae were absent in the first segment (stage B). Rudiments of tentacles were observed on the 15th day (stage II) From the 17th to the 26th day a decrease in weight from 302 mg to 248 mg (= 15%) occured. During the same period the oxygen consumption increased to 130 cu.mm (on the 22nd day) and 135 cu.mm (on the 26th day).
The morphological changes during this time

FRAGMENT 33.

FIG. 18.



were the complete loss of setae in 4 segments, the appearance of new setae (on the 18th day in 2 segments and on the 23rd day in 4 segments) and new uncini (beginning with the 26th day). The regeneration reached stage III on the 21st and stage IV on the 27th day.

Between the 26th day and the end of reorganization (stage D on the 35th day) and regeneration (stage V on the 43rd day), weight and respiration were slowly decreasing.

The total loss of weight during the period of regeneration and reorganization was 35 mg (268 mg on the 2nd, 233 mg on the 44th day) i.e. ca. 13%.

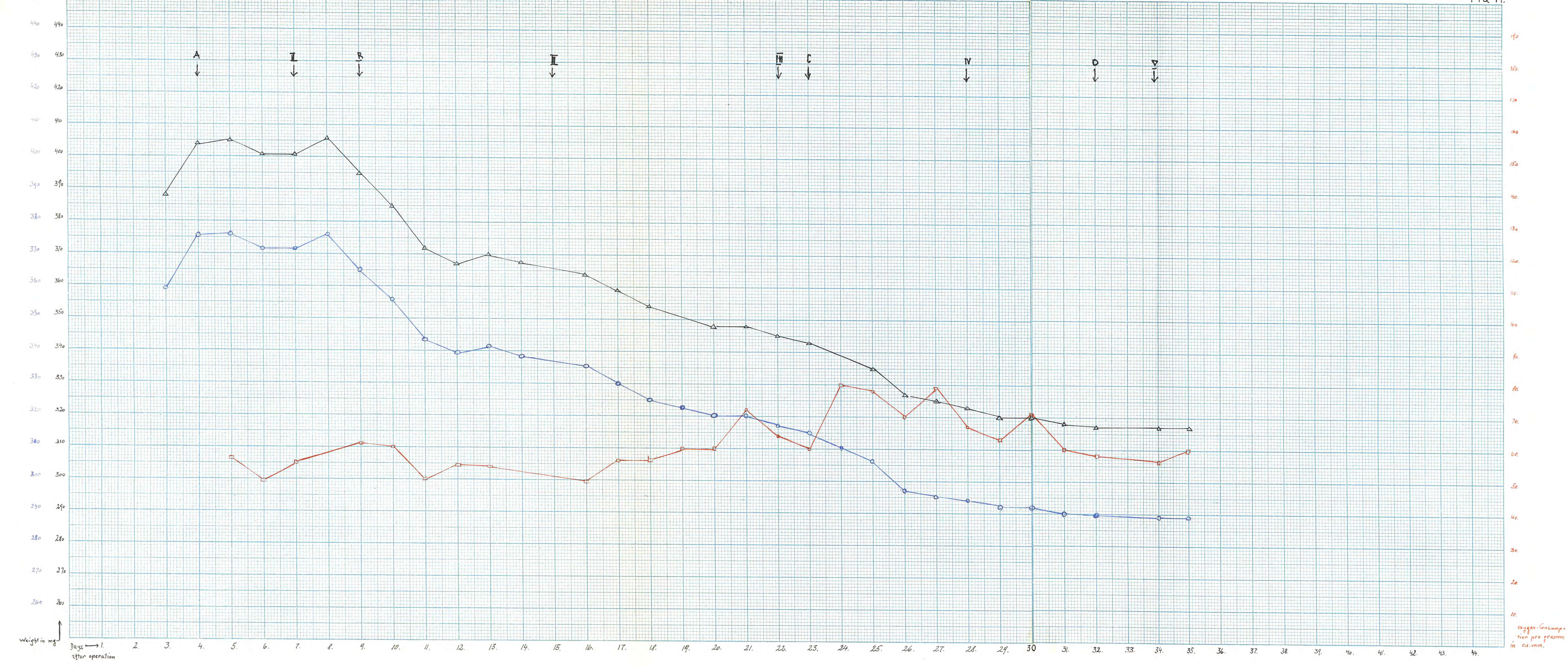
The difference between maximal weight (302 mg on the 18th day and weight after regeneration was 69 mg, i.e. ca. 23%.

Fragment 33 (Fig. 18) behaved in a very similar way. It consisted of 35 segments and weighed 270.5 mg on the second day after cutting. The first signs of regeneration appeared on the 7th day, uncini were dropped in 1 segment on the 8th day, in 3 segments on the 15th day, when also the first rudiments of the tentacles were observed. Uncini were lost in 4 segments on the 17th day and setae in 1 segment on the same day.

During that period the weight , after an ini-

FRAGMENT 37.

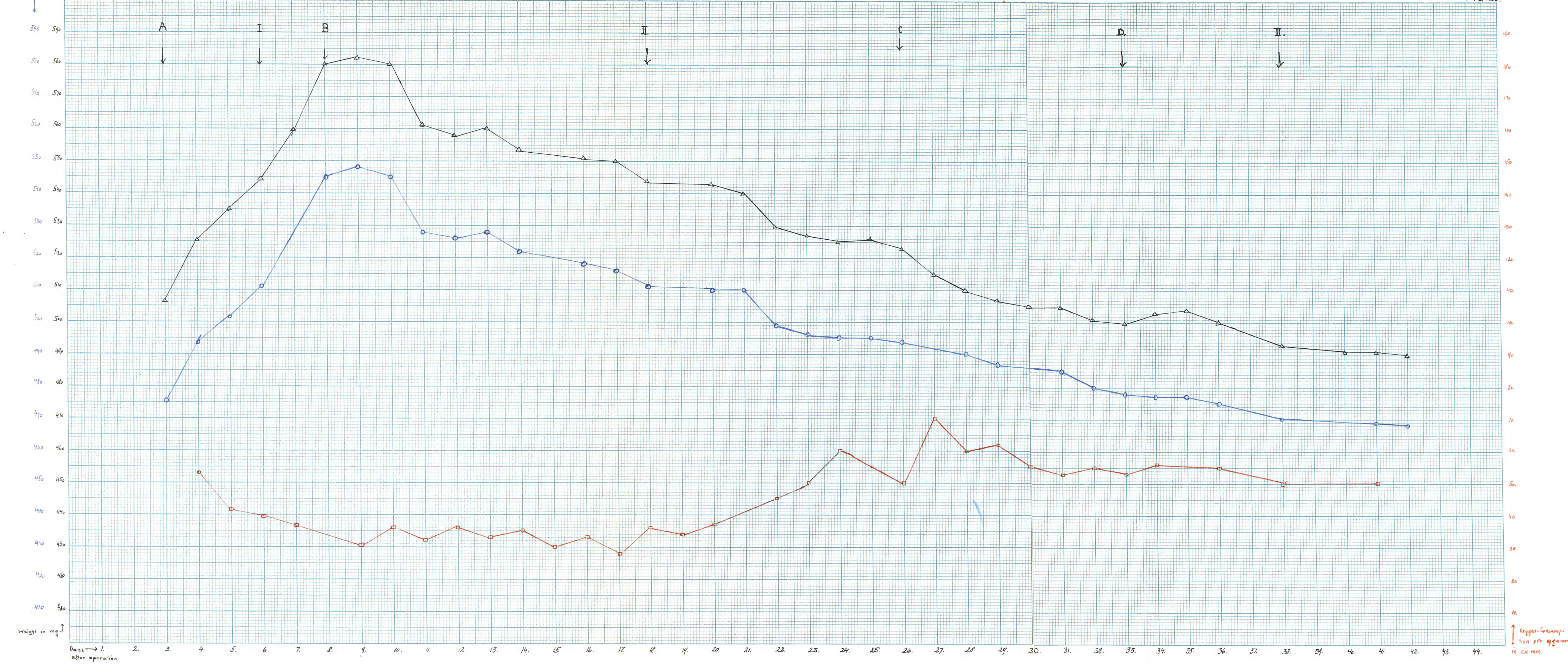
FIG 19.



tial drop from 27o mg on the 2nd day to 260 mg on the 7th day increased to 295 mg on the 20th day.
During the next 10 days the weight fell to 236 mg; setae were lost in 3 further ~~further~~ segments and new setae were formed; as regards regeneration stages III and IV were reached on the 25th and the 29th day respectively.
The respiration, after a decrease from 64 cu.mm on the 4th day to 32 cu.mm on the 15th day, increased steadily and reached 100 cu.mm on the 24th day; after a further drop to 74 cu.mm it increased again to a maximum of 109.1 cu.mm per gramm/hour.
Subsequently weight and respiration decreased slowly before stage V of regeneration and stage D. of reorganization were reached.
The total loss of weight between the 2nd and the 45th day was 45.5 mg i.e. ca 20%; between the maximum and the end point 70 mg or roughly 25%.
In fragment 37 (Fig. 19), 39 segments;, operated 1o.4.1939; the loss of weight during the period of experiment was less than 20% (388 mg on the 3rd day to 328 mg on the 35th day) and the difference between the maximum (406 mg on the 8th day) to the endpoint (35th day) amounted to barely 20%.
The oxygen consumption rose during the ex-

FRAGMENT 32.

FIG. 20.



periment from 56.7 cu.mm on the 5th day to 80.3 cu.mm and 79,1 cu.mm on the 24th and 27th day respectively, and fell to 60 cu. mm on the 35th day.

3 segments had been reorganized.

An extreme was reached in

fragment 32 (Fig. 20), operated 9.3.1939; 48 segments; the weight was 508.5 mg on the third day and rose steeply to 582 mg on the 9th day. The following decrease was slow, and the usual sharp decline in weight was confined to one day and amounted to only 20 mg. The total loss of weight in 39 days amounted to 16.5 mg or 3%, the loss between maximal weight and weight after reorganization to 82 mg or 17%.

Respiration decreased from 53 cu.mm on the 4th day to 30,4 cu.mm on the 9th day. After that it remained more or less constant around 35 cu.mm and rose between the 20th and 24th day to 60 cu.mm and further to 70 cu.mm (on the 27th day). Finally it decreased to 50 cu.mm on the 41st day.

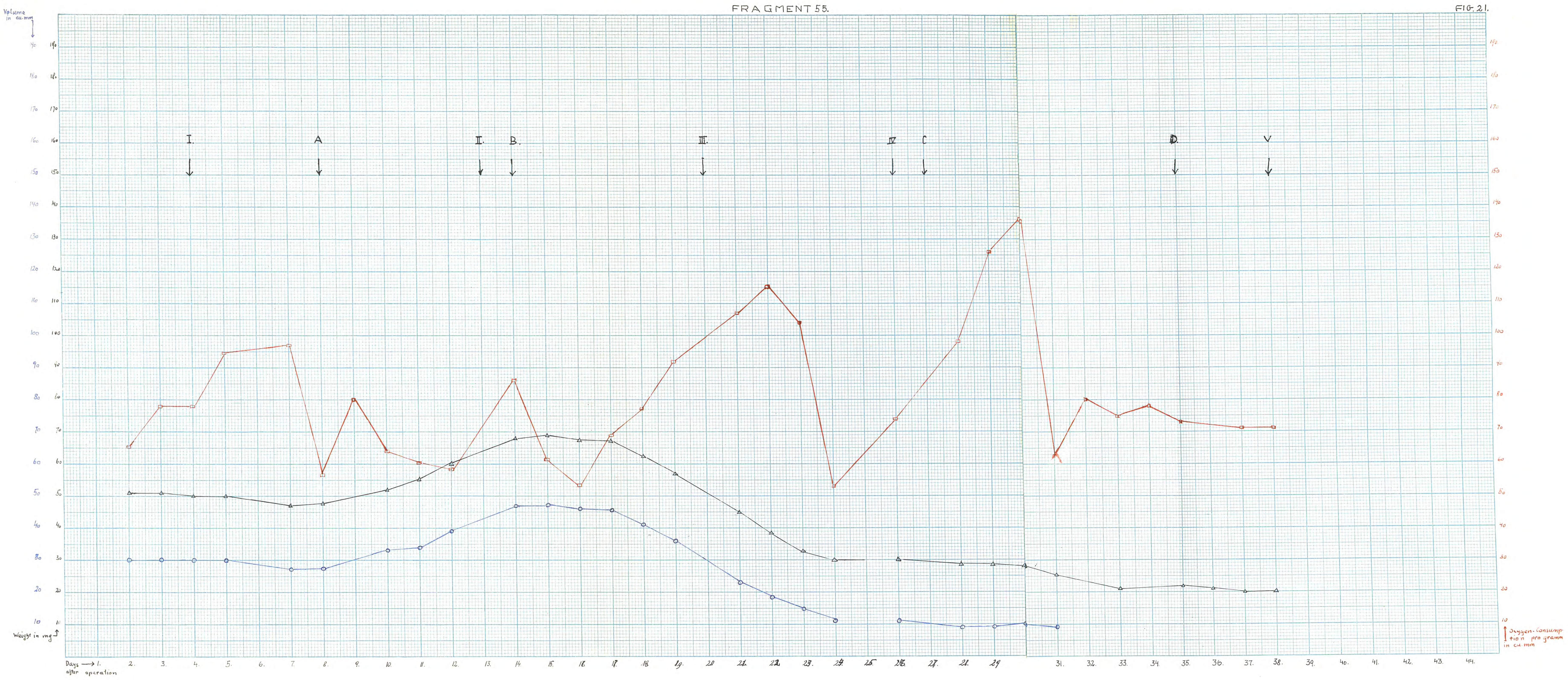
Only two segments had been reorganized at the end of the experiment and regeneration only reached stage III. after 41 days.

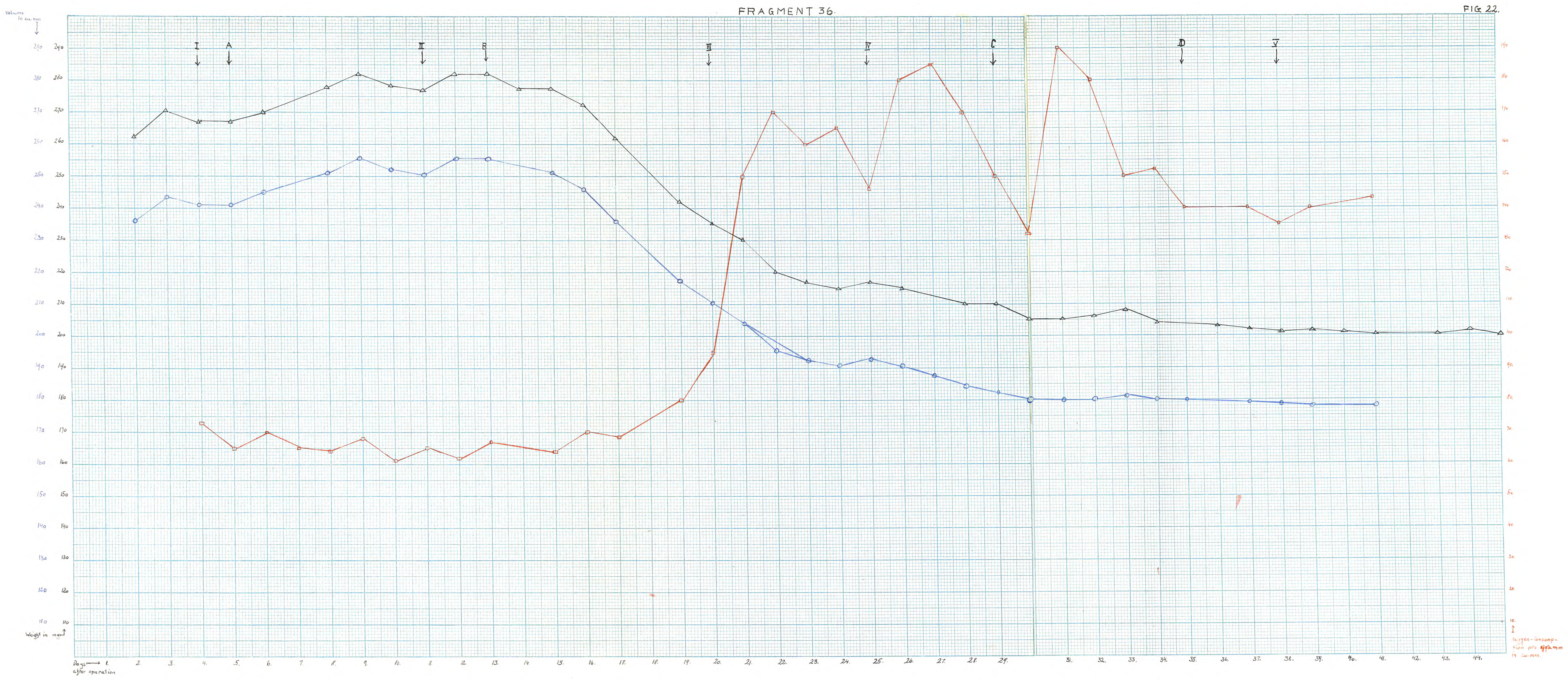
It may be added that fragments 37 and 32 were the most anterior parts of the abdominal region; they passed through the earlier stages of reorganization more rapidly than fragments 33 and 40 belonging to more posterior regions.

b.) Amputation Experiments.

A series of experiments will be described now, in which after complete reorganization fragments were amputated again. As can be seen from Figs. 10, 11, 12 etc. respiration of fragments remained, even after completion of reorganization, much higher than shortly after cutting. It was therefore of interest to find out how the oxygen consumption of amputated fragments, i.e. of fragments the thoracic region of which was removed after complete regeneration and reorganization, compared with their oxygen consumption previous to the amputation. Another point of interest was to see if such amputated fragments would show the same behavior as fragments from anterior abdominal parts. (pp. 15/16).

Six experiments were started but only three fragments remained alive until regeneration was complete. The results obtained with these specimens were very uniform and fragment 55 may be taken as representativ and described ~~more~~ in detail.


FRAGMENT 55.
FIG. 21.
Volume in cu.mm
Weight in mg
Days after operation
Oxygen-consumption pro gramm in cu.mm
I.
A
II.
B.
III.
IV
C
D.
V



FRAGMENT 36.
FIG. 22.
Volume in cu. mm
Weight in mg.
Days after operation
I
A
II
B
III
IV
C
D
V
Oxygen-Consumption pro gramm in cu. mm.

Fragment 55 (Fig. 21) was derived from Fragment 36 (Fig. 22); 32 segments. It was cut 5 days after complete reorganization and regeneration. The weight before cutting was 98 mg, the oxygen consumption 131.4 cu.mm. 5 segments had been reorganized.

The head region, the thorax and two abdominal segments were removed and 25 segments kept after cutting.

The weight 2 days after cutting was 51 mg and the respiration went down to 65.6 cu.mm i.e. approximately the same amount as in the initial stages during the first period of reorganization.

As shown in fig 21 the development was similar to that of posterior fragments. Stages A. and B. were reached only slowly, in fact more slowly than in the original fragment. The weight also increased slowly and reached the maximum only between the 14th and 17th day. But the increase in weight was very remarkable. It rose from 51 mg on the 2nd to 69 mg on the 15th day, more than 30% of the original weight. Such a comparatively high gain in weight was never observed in any fragment cut for the first time, and increases in weight in small fragments never reached nearly as high a percentage as in these three fragments after amputation. The oxygen consumption fell after amputation to the normal ratio ~~and~~ but it was not observed, if this decline ~~was~~ occurred directly after cutting or some time later. The metabolic changes appeared to have been on the whole more energetic than in the original fragment, but the maxima were lower. The number of segments reorganized was 5 in fragment 55 i.e. the same number as in the original fragment; in one other fragment it was also the same (6) and in the third, one segment more was affected by reorganization (7 altogether).

The results described above show that there are distinct phases in the change of weight and oxygen consumption, which correspond to simultaneous morphological changes in the fragments.

The first phase, in which early signs of regeneration may appear (stage I) is characterised by a slow fall or a stability in weight.

The second phase leading up to stage B, in which reorganization begins with the loss of uncini and the degeneration of the uncinigerous lobes, is characterised by a more or less high increase in weight, which may reach a level higher than the original. During this period the respiration is more or less constant around the initial intensity.

The third phase, leading up to stage C. in which very energetic changes take place, involving the loss of setae and the degeneration of setigerous lobes on the one hand and the formation of new setae as well as the differentiation of the head region on the other hand, is characterised by

1) a sudden and steep decrease in weight (sometimes up to 60%) and
2) an equally steep rise in the oxygen consumption.

But while the decrease in weight is slowed down after a period, which is usually correlated with the end of the loss of setae, the oxygen consumption remains on a very high level during the following stages of regeneration and reorganization and decreases only after the end of the process.

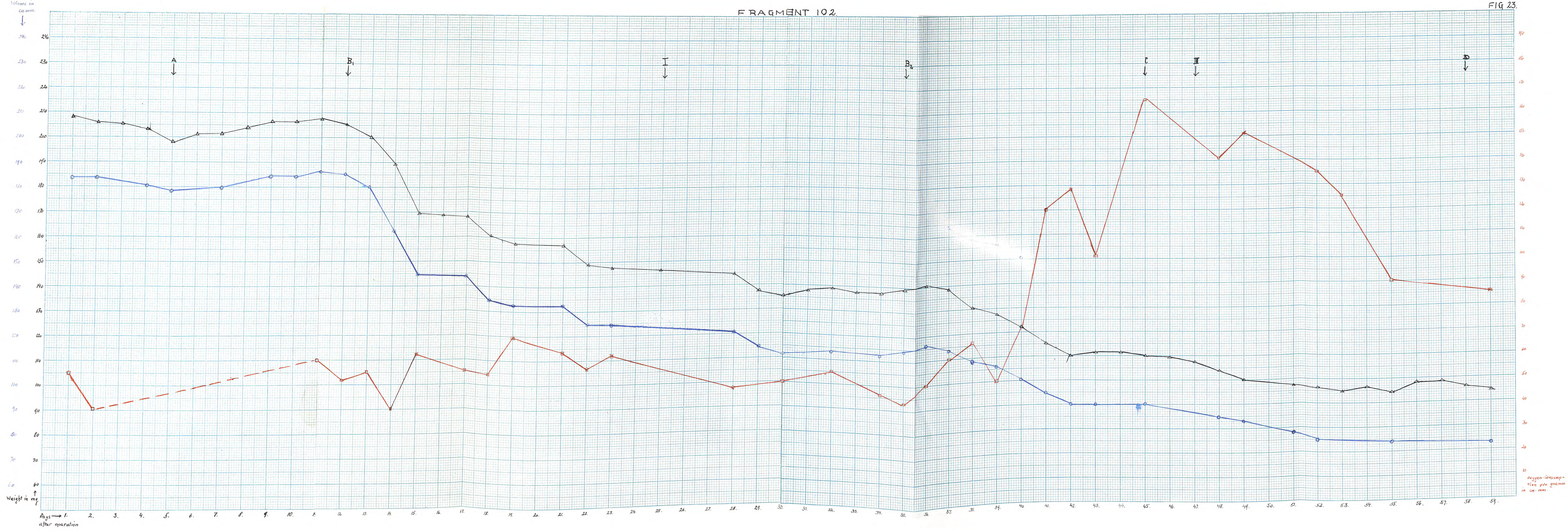
Gross and Huxley (1935) had pointed out that there are two different phases in reorganization: a degenerative and a reconstitutive phase. At room temperature these two phases partly overlap each other, as new setae appear at the same time as old ones are being lost.

From the diagrams, shown so far, it can be seen that two distinct processes take place simultaneously, namely the decrease in weight and the increase in the oxygen consumption. It was thought that the two processes were linked up and that the increase in respiration involving oxidation of an increasing amount of body substances naturally leads to a faster decrease in weight. This seemed to be confirmed by the fact that the weight of setae lost, never amounted to more than a fraction of the weight lost. The weight of setae from 5 segments was found to be 2.5 mg.

But one fragment was found (fragment 32, Fig. 20) in which a very distinct independence of the two processes was noted. This fragment, amongst other pecularities discussed earlier in this paper (p. 20), shows the rapid decrease clearly separated from the increase in the oxygen consumption. It was also found that the loss of setae and the appearance of new ones was distinctly separated in this fragment (loss of setae in two segments between the 8th and 11th day, appearance of new ones on the 22nd day) and the regeneration was extremely slow (not finished after 41 days).

Gross and Huxley (1935) have pointed out that a very sharp separation in time of the degenerative and reconstitutive phases could be observed in fragments kept at low temperature, (8° - 10°C) It was therefore thought that frag-

FIG. 23.



FRAGMENT 102
Volume in cu.mm.
Weight in mg.
Days after operation
A
B1
I
B2
C
II
D
oxygen-consumption pro gramm in cu.mm.

ments kept at low temperature might show more clearly the correlation between the morphological changes and those of weight, volume and oxygen consumption.

c) Experiments at low temperature.

An unheated room in winter was used for keeping the fragments at low temperature and unfortunately a sudden fall of temperature below ~~the~~ freezing point killed off 4 out of a series of 6 fragments under investigation.

But the results obtained in the two fragment left were very interesting and uniform and are shown in Fig. 23, representing the processes of reorganization and regeneration in

Fragment 102. In this specimen a very clear separation of the degenerative and reconstitutive phase could be established; the loss of uncini (stage A) and the loss of setae (indicated as stage B_1) occurred far in advance of the appearance of new setae (indicated as B_2) and uncini (stage C). The first stages took place around the 5th and the 12th day respectively, the latter around the 34th and 46th day. Regeneration was very much delayed and not completed at the end of reorganization.

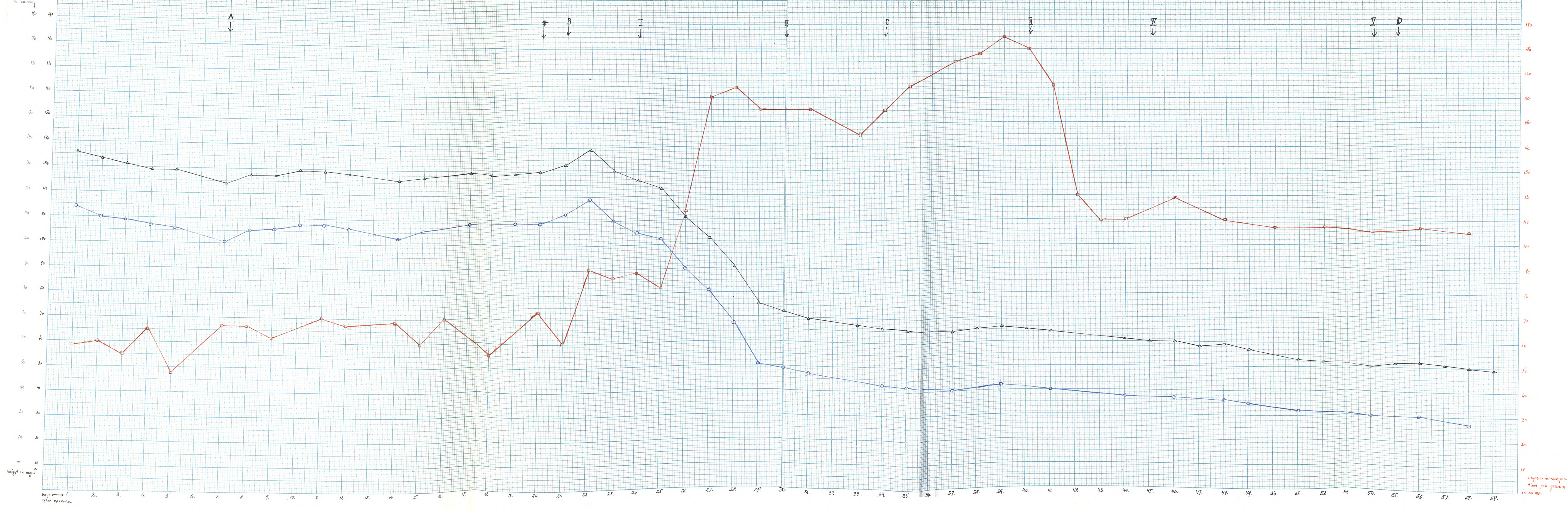
The sequence of the morphological and physiological changes was as follows: in the first stage (A) there was no difference compared with the fragments kept at room temperature except for the slower speed of development. The subsequent decrease in weight, correlated with the loss of setae and the formation of new ones in earlier described fragments (stage B-C) was clearly separated into two phases. The first followed stage B_1 (loss of setae) with a fall from 207.5 mg to 170 mg, and the second stage B_2 (formation of new setae) with a fall from 140 mg on the 36th day to 110 mg on the 42nd day. In this second stage, belonging entirely to the reconstitutive phase the oxygen consumption rose - after a period of comparative stability - steeply from 50 cu.mm to 128 cu.mm and after a fall to 101,2 cu.mm on the 43rd day again to 165,1 cu.mm on the 46th day (stage C).

Between the stages III of regeneration and D of reorganisation the oxygen consumption decreased again.

An examination of Fig. 23 gives the impression of the c~~o~~urve having become separated into two units characterising two phases following one another. ~~The~~ One signifies the loss and degeneration of old material, the other the formation of new ~~one~~. The slope of the c~~o~~urve is more or less similar

FRAGMENT 153

FIG 28.



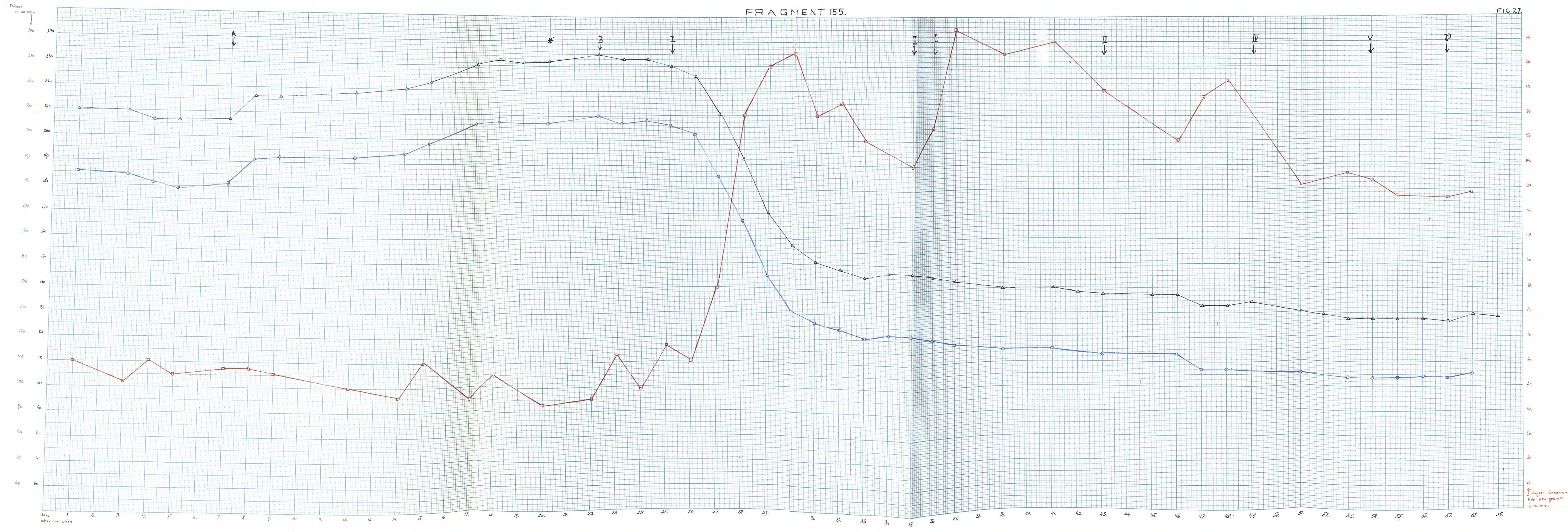
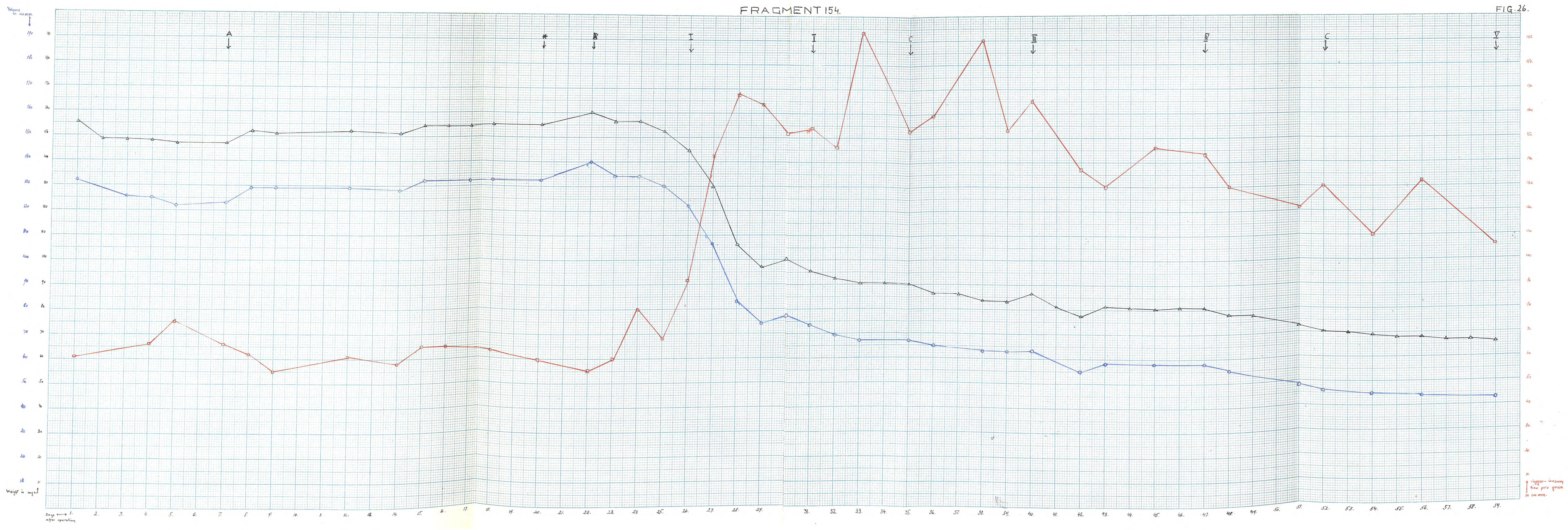


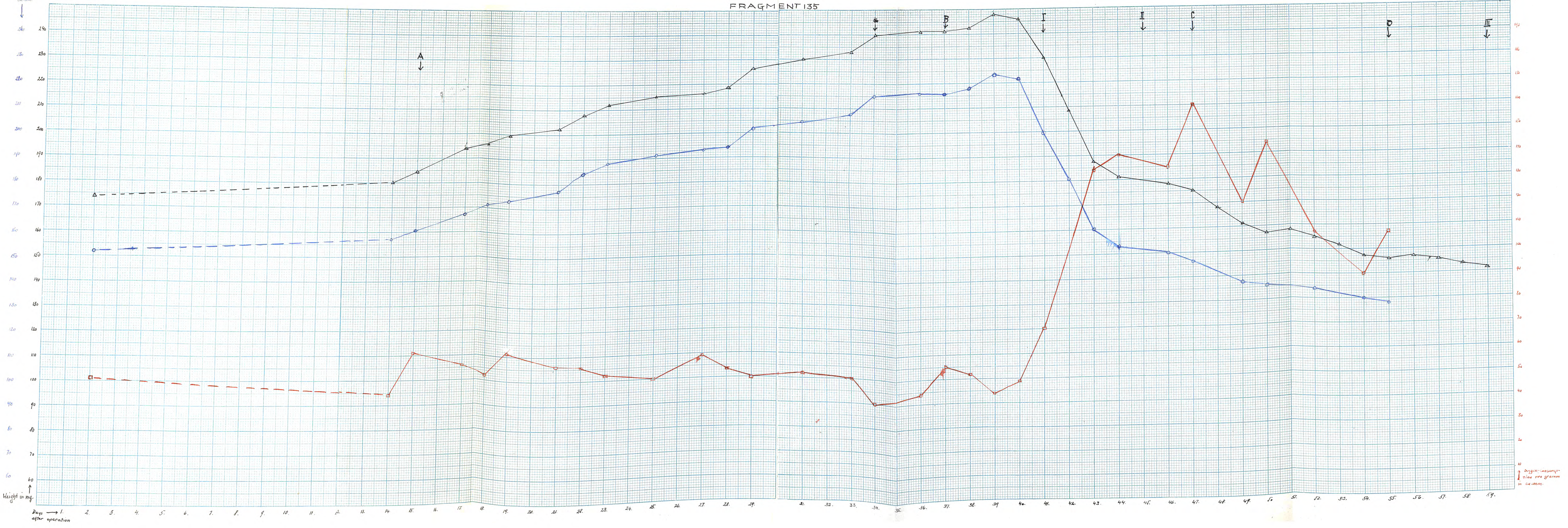
FIG 27.

FRAGMENT 155.



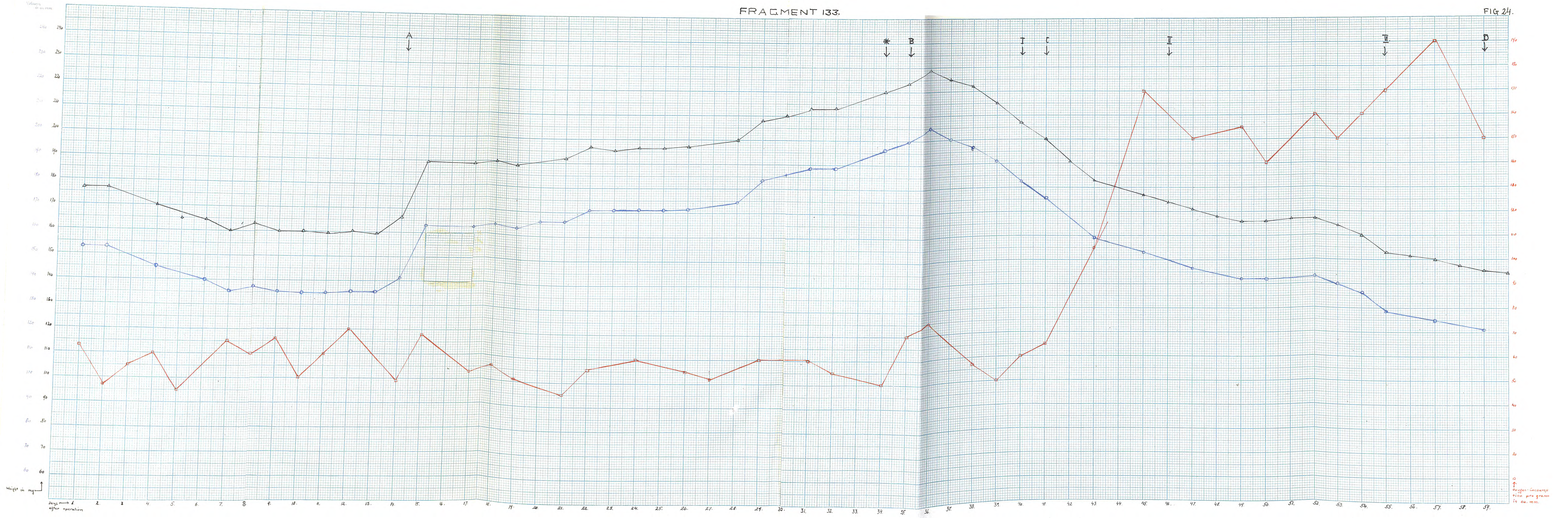
FRAGMENT 154.
FIG. 26.
A
*
B
I
II
C
III
IV
C
V
Days after operation
Weight in mg.

FIG. 25.


FRAGMENT 135
Volume in cu.mm.
Weight in mg.
Days after operation
Oxygen-consumption pro gramm in cu.mm.
A
B
C
D
I
II
III

FRAGMENT 133.

FIG 24.



in both phases. The rise in the oxygen consumption on the other hand belongs exclusively to the second, the reconstitutive phase.

This seems to indicate that in fragments, kept at room temperature, the steep and more or less uniform decline in weight may possibly represent two different successive phases of development with different physiological significance.

d) Change of temperature.

The following five fragments (Figs. 24, 25, 26, 27, 28.) belong to a series of experiments in which the temperature was changed from low temperature to room temperature in the course of experiment.

This change took place after loss of uncini and before any sign of regeneration could be observed. The day, when the fragments were transferred from low temperature (6^{o}–8^{o} C.) to room temperature (18^{o}– 20^{o} C.) is indicated in the diagramms with an asterisk (*).

The figures show that a day or two after changing the temperature the setae were lost, and that the course of reorganization which followed was very similar to that normally observed at room temperature.

It appeared however that reorganization proceeded more rapidly after change to room temperature in fragments which had been kept at low temperature for a comparatively long period; Fragments 133 and 135, 34 days, compared with Fragments 153, 154 and 155, which had been under the influence of low temperature for a shorter period (20 days). Moreover regeneration was very much more delayed in the first as compared with the latter instances.

Discussion.

The experiments described show that in Sabella there exists a correlation between the morphological changes during regeneration and reorganization on the one hand, and changes in weight, volume and oxygen consumption of fragments on the other.

Regenerating fragments are incapable of taking in any food prior to the completion of the head region. Moreover, the medium in which they have been kept was practically sterile. They must have therefore necessarily used up a certain quantity of body substance for the maintenance of life and particularly for the regenerative processes. From this consideration one should expect a steady decrease in weight in the course of regeneration.

It is therefore remarkable that one period of morphological changes is accompanied by a more or less marked increase in weight. This can only be caused by the absorption of water and perhaps of some substance present in solution in the surrounding seawater. That the diffusion takes place through the whole surface of the fragment and not only in the region affected by the reorganization is shown by the fact that the increase in weight is generally higher in larger fragments with a larger surface*, and by the increase in volume accompanying the increase in weight shown in all diagrams of regenerating fragments. ?

It may be assumed that right from the beginning of wound-healing and regeneration a certain amount of body substance is being used up. The resulting decrease in weight is, however, masked by the incorporation of water during the first phases of regeneration and reorganization, when the changes taking place are not very drastic.

In this connection attention may be drawn to the marked decrease in density of frog eggs in the

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* Only in one instance, referred to above (p. 17) a very small fragment after amputation showed a comparatively high increase in weight. The significance of this fact will be discussed later.

early stages of embryological development. Briggs (1939) in a recent paper suggests that "as development proceeds yolk is transformed gradually into protoplasm of much higher water content, the water required in this transformation being absorbed largely from the medium. The metabolism of yolk probably involves a breakdown of large molecules or molecular complexes into smaller ones with consequent increase in osmotic pressure and absorption of water."

It might be tentatively suggested that during the processes of reorganization, particularly during the early degenerative phase, the breakdown of material required for the formation of the regeneration causes a similar increase in osmotic pressure and sonsequently absorption of water.

During the next period when setae are lost and setigerous lobes resorbed, as well as when new setae and setigerous lobes are formed the behaviour of the regenerating fragment becomes different.

As was shown in fragments kept at low temperature, the following decrease in weight is correlated with two clearly separated phases: firstly, the loss of setae and the resorption of the setigerous lobes, and secondly, the formation of new setae and setigerous lobes.

The first phase is characterised by a considerable loss in weight without any marked change in respiration and the second by a very high increase in oxygen consumption. During both phases the weight decreases considerably, but during the first, respiration remains at about the same level, while during the second the oxygen consumption rises very markedly and rapidly.

It seems likely that during the first phase very little, if any, water is absorbed and that the marked drop in weight indicates the real extent of drainage upon reserve substances and tissues.

With regard to the second phase it is possible that the increased respiration indicates that more reserve substances and products of histolysis have been oxidized than would appear from the relatively small decrease in weight during the phase.

It is remarkable that between the stages C-D in the reconstitutive phase and after completion of reorganization and regeneration the high oxygen consumption does not affect the weight, which is decreasing very slowly. It may be suggested, therefore, that during the formation of new setae as well as during the subsequent phases of regeneration, a certain amount of water was absorbed, which masked the constant decrease in weight of the fragment.

From this suggestion it would follow that during the period of regeneration and reorganization a considerable absorption of water takes place - (1) during the initial "degenerative phase", and (2) during the reconstruction phase, the first being probably concerned with the formation of the regenerating blastema, the second with the formation of new setae and uncini.

In this connection reference may be made to Fragment 55 (p.). At the beginning of regeneration and reorganization an extraordinary increase in weight (30% of the weight of the fragment after amputation) took place, while the rate of respiration was lower than before amputation, and this seems to indicate that the tissues were still capable of absorbing as much water as in the period of formation of new uncini and after completion of reorganization. This supports the assumption that the decrease in weight during the stages of reorganization and regeneration, caused by very high respiration, is masked by absorption of a great amount of water.

The first attempt to explain the phenomenon of reorganization of abdominal segments into thoracic ones in Sabella was made by Berrill (1931) who considered that the regenerating head acted as an "organiser". He states that this "organiser" exists in the anterior regenerated tissue, that its force suffers a decrement as it entends posteriorly, that its nature is electrical or neuroid rather than hormonal or due to diffusion of agents..."

But several facts, described by Gross and Huxley (1935), negativated Berill's suggestion. These facts were: the existence of posterior reorganization; the appearance of signs of re-organization before regeneration; the occurrence of reorganization without regeneration and reversal in the direction of reorganization following upon surface wounds. According to Gross and Huxley reorganization appears to be an example of "alternative differentiation". While the thoracic segments are unable to undergo further differentiation, abdominal segments, being more primitive and plastic, are capable of transformation into segments of thoracic type, thus leading again to a stage of irreversible determination and differentiation. They distinguish (1) a degenerative and (2) a reconstitutive phase of reorganization and suggest that during the first phase material is transported from the segments in the neighbourhood of the cut to the cut surfaces themselves. This removal of material causes the disorganization and degeneration of the parapodia of those segments, and leads to their transformation into thoracic segments ("reconstitutive phase").

Berrill (1936 b.) in a later paper maintains against the views of Gross and Huxley that there is no direct proof of a cell-migration; that even assuming that - in analogy with other Polychaetes - such may be the case in Sabella, it can not account for degeneration in such a large number of segments as 78; and that, finally, the summarizing effect of repeated decapitation makes it highly improbable. He puts forward the view that a cut of any kind tends to establish conditions inducing thoracic organization, but that normally the formation of a pygidium and posterior zone of growth inhibits its appearance in posterior abdominal segments. Berrill describes observations regarding an increase in number of reorganized segments through the influence of light and suggests that "the process or activity initiated by a cut... is of such a nature that its activity or intensity is augmented either as a photo-chemical reaction or by the products of a photo-chemical reaction." He concludes that this takes place through "the liberation of electrical energy rather than by the diffusion of a chemical evocator."

The results reported in this paper support the distinction between a degenerative phase, coinciding with the formation of rudiments of the anterior regenerates (head region), and a reconstitutive phase which marks the formation of appendages of thoracic type. It could be shown that the physiological behaviour of the fragments as expressed in changes of weight, volume and oxygen consumption was different during the two phases and that, in particular, metabolic activity as indicated by O_2 consumption was very much higher during the second phase than during the first.

The most anterior part of the abdominal region tended to complete the destructive phase earlier than more posterior fragments under the same external conditions. If we assume that the cut surface can initiate reorganization by the liberation of energy of some sort we would have to conclude that the energy varied in amount and intensity in different fragments, and was more effective in fragments from the region behind the thorax. But the behaviour of fragments whose thorax was amputated after a completed regeneration (36, 55, p...) do not bear out such a conclusion. The speed of regeneration and reorganization in the first phase (A, B, I, II) was not higher than in the fragment before amputation.

As to the ratio of segments reorganized, it was found that heavier fragments tend to reorganize a smaller number of segments. For example of ~~fragments~~ fragment 32 only 2 segments out of 48 were reorganized. This fragment was an anterior part and the phase of loss of uncini and setae was a very short one. On the assumption that "transmission of energy" of some sort is responsible for reorganization it is difficult to see why the effect of this agent, causing such rapid changes should be confined to only 2 segments.

Since the ~~universe~~ increase in weight was probably caused by intake of water it may be that the speeding up of the degeneration phase in fragments from the anterior abdominal region is correlated with the imbibition of larger quantities of water by the tissues.

Gross and Huxley's observations that low temperature has a different effect on regeneration and reorganization were confirmed (see fragments 133, 135, 153, 154, 155, p.24); for it would appear that regeneration is greatly slowed down, the more so, the longer the fragment is kept at low temparature, while the degenerative phase of reorganization was not affected to the same extent.

These facts were interpreted by the authors referred to on the assumption that two distinct processes are at work - cell-division and cell-migration, - of which the first is more inhibited by cold than the latter. By cell-migration they understood the movement of cells over the cut surface and the immigration of cells of various types into the regeneration blasteme; "this immigration may affect several segments back from the cut and may then cause important physiological changes such as loosening of the tissues, active histolysis etc.; these in turn would cause the initiation of the degenerative phase of reorganization". Cell-division, they assume, is more involved in posterior regeneration.

It is also possible that instead of, or combined with cell-migration, transportation of non-cellular-material, protein and sugar molecules, takes place from the segments behind the cut towards the cut surface. However this may be, there is reason to assume that the degenerative phase of reorganization - coinciding with or preceding the formation of the first rudiments of head segments - is based on physiological processes which involve no considerable increase in oxidation and are not strongly affected by cold. On the other hand the reconstitutive phase and the growth of head and tail segments involve an extremely high rate of oxygen consumption and are strongly affected by cold.

Summary

1) During the first period of regeneration and reorganization of fragments from the abdominal region of *Sabella*, marked by loss of uncini, their weight and volume increase during the period in which reorganization is started, and respiration remains more or less unchanged.

It is suggested that the increase in weight and volume is due to the taking up of water.

2) During the succeeding period, marked by loss and degeneration of setae, and formation of new setae (at room temperature) weight and volume decrease considerably and rapidly while respiration increases markedly.

3) A third period consists of two phases, distinctly separated at lower temparatures; the first characterized by a decrease of weight without increase of respiration, coinciding with the degenerative processes; the second by loss of weight with a sharp increase in the rate of oxygen consumption, coinciding with the formation of new appendages.

4) Anterior fragments from the abdominal region seem to pass more quickly through the degeneration phase. They also show a greater initial increase in weight than fragments from more posterior regions.

5) Fragments amputated after a complete regeneration and reorganization had taken place do not show that rapid degeneration of original appendages, shown by fragments mentioned in (4), but they exhibit a high increase in weight in the early stage of reorganization.

6) Regeneration was found to be markedly delayed by exposure to low temparature, and this effect increased with the length of time of exposure. Reorganization also is delayed but not to the same extent, - and after transference to room temperature the process is rapidly completed.

7) These results and their bearing on the interpretation of the phenomena of regeneration and reorganization are discussed

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